Evidence of Detrimental Effects of Environmental Contaminants on Growth and Reproductive Physiology of White Sturgeon in Impounded Areas of the Columbia River

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Abbreviations:

PCB Polychlorinated Biphenyls

p,p’-DDT para para Dichlorodiphenyltrichloroethane

p,p’-DDE para para Dichlorodiphenyldichloroethylene

p,p’-DDD para para 1,1-dichloro-2,2-bis(4-chlorophenyl)ethane

GSI Gonadosomatic index

E2 17β-estradiol

T Testosterone

KT 11-ketotestosterone

Vtg Vitellogenin

Ca Calcium

TAG Triacylglycerides

RIA Radioimmunoassay

MA Macrophage aggregates

v/v Volume to volume

Ni Nickel

Tris Tris(Hydroxymethyl)aminomethane

KCl Potassium chloride

EDTA Ethylenediaminetetraacetic acid

SDS-PAGE Sodium dodecyl sulfate polyacrylamide gel electrophoresis

ANOVA Analysis of variance

EDCs endocrine disrupting chemicals

Outline of manuscript

Abstract

This study sought to determine if wild white sturgeon from the Columbia River (Oregon, USA) were exhibiting signs of reproductive endocrine disruption. Fish were sampled in the free-flowing portion of the river (where the population is experiencing reproductive success) and from 3 reservoirs behind hydroelectric dams (where fish have reduced reproductive success). All of the 18 pesticides and almost all of the 28 PCBs that were analyzed in livers and gonads were detected in at least some of the tissue samples. Metabolites of p,p'-DDT (p,p'-DDE and p,p'-DDD) were consistently found in fish at relatively high levels. Some males and immature females showed elevated plasma vitellogenin; however, concentrations were not correlated with any of the pesticides or PCBs analyzed. Negative correlations were found between a number of physiological parameters and tissue burdens of toxicants. Plasma triglycerides and condition factor were negatively correlated with total DDT (DDD + DDE + DDT), total pesticides (all pesticides detected – total DDT) and PCBs. In males, plasma androgens and gonad size was negatively correlated with total DDT, total pesticides and PCBs. Fish residing in the reservoir behind the oldest dam had the highest contaminant loads and incidence of gonadal abnormalities, and the lowest triglycerides, condition factor, gonad size and plasma androgens. These data suggest that endocrine disrupting chemicals may be accumulating behind dams over time. Overall, results of this study indicate that exposure to environmental contaminants may be affecting both growth and reproductive physiology of sturgeon in some areas of the Columbia River.

Introduction

The lower Columbia River supports one of the most productive white sturgeon, *Acipenser transmontanus*, fisheries in North America (DeVore et al. 1995; McCabe and Tracy 1994). Fish trapped behind the dams of the hydroelectric system however, have reduced reproductive success when compared to animals in the free-flowing portion of the river (Beamesderfer et al. 1995). Reduced reproductive fitness of fish in these impounded sections of the river has been attributed to habitat, flow, and temperature, but environmental toxicants could also be playing a role. The long-lived, late-maturing and benthic lifestyle of sturgeon may make them particularly susceptible to the actions of persistent bioaccumulating pollutants (DeVore et al. 1995).

The Columbia River receives pollution from a variety of sources that include sewage treatment plants, bleached-kraft pulp mills, aluminum smelters, mining operations and agricultural and urban runoff. Recently, it has been determined that past operation of the hydroelectric facilities has led to contamination of certain areas of the river with PCBs (URS Corp. 2002). A wide variety of environmental contaminants have been shown to have adverse effects on reproduction in fishes (Tyler et al.1998; Van Der Kraak 1998; Kime 1995), and many of these bioaccumulating toxicants have been detected in sediments and fish from the Columbia River (EPA 2002; Foster et al. 1999, 2001a, 2001b).

This study was designed to examine whether environmental pollutants are having an adverse effect on the reproductive physiology of white sturgeon in the wild and to determine if fish demonstrate evidence of reproductive endocrine disruption that correlates to specific areas within the river where sturgeon are known to have low reproductive success.

Materials and Methods

Fish sampling

Fish were sampled during the commercial and sport harvest in February-April of 2000 and 2001. Due to state fishing regulations only fish within a slot limit of 110-137 cm fork length were sampled. This slot limit is set to ensure that mature fish are not removed from the fishery. Fish were sampled from four areas of the Columbia River: the free-flowing portion of the river in the estuary at Astoria, Oregon (OR) and in reservoirs above Bonneville (river mile 191), The Dalles (river mile 216) and John Day (river mile 292) dams (Figure 1). These dams were constructed in 1938, 1960 and 1971, respectively. A total of 174 fish were sampled representing 42-45 individuals (19-24 males and 21-23 females) for each location. Length and weight were recorded and condition factor was determined. Gonads were removed, weighed and gonadosomatic index (GSI) was determined. Gonads and livers were collected for both histological and contaminant analysis. Plasma samples were collected for analysis of 17β-estradiol (E2), testosterone (T), 11-ketotestosterone (KT), vitellogenin (Vtg), calcium (Ca), and triacylglycerides (TAG). In 2001, pectoral fin spines were collected to determine the age of fish. All animals were treated in accordance with Oregon State University's Care of Laboratory Animals guidelines.

Plasma analyses

The steroids T, KT and E2 were extracted from plasma following the method of Fitzpatrick et al. (1986). Extraction efficiencies for all steroids were determined by adding tritiated steroids to tubes containing plasma (n=4) during each extraction. This resulted in 12 extraction efficiencies for each steroid. The average extraction efficiencies and (ranges) for T,

KT and E2 were 92.5 (88.8-94.6), 82.5 (81.6-83.0) and 83.4% (79.8-85.5%), respectively. All steroid assay results were corrected for recovery.

Plasma concentrations of T, KT and E2 were measured by radioimmunoassay (RIA) as described in Sower and Schreck (1982) and modified by Feist et al. (1990). All samples were analyzed in duplicate. The lower limit of detection was 1.25 pg/tube for all assays, except KT (3.12 pg/tube). The intra- and inter-assay coefficients of variation for all assays were less than 5 (n=12) and 10% (n=12), respectively. Steroid levels determined by RIA were validated by verifying that serial dilutions were parallel to standard curves.

Vitellogenin was measured by enzyme immunoassay following the methodology of Linares-Casenave (1994) and Heppell and Sullivan (1999). Purified white sturgeon Vtg and antibody were a gift from Dr. S. Doroshov (University California-Davis). The lower limit of detection was 3.9 ng/ml and the assay was validated by verifying that serial dilutions of samples were parallel to the standard curve. The intra- and inter-assay coefficients of variation were less than 5 (n=72) and 10% (n=72), respectively. Calcium and TAG plasma content were determined using diagnostic kits from Sigma (587-A and 334-A).

Histology

Gonad and liver tissue was stored in 10% phosphate-buffered formalin, embedded in paraffin, sectioned at seven µm, and stained by hematoxylin and eosin (Luna 1968). Slides were examined under a compound scope (Motic, 10x-100x). Germ cells were scored for stage of development according to the protocol of Van Eenennaam and Doroshov (1998). Stage 1 (differentiation of testis and ovary) and Stage 2 (proliferation of spermatogonia and endogenous growth of the oocyte) fish were immature, while Stages 3 - 6 males (onset of meiosis through

spermiation) and Stages 3 - 7 females (early vitellogenesis through ovulation) were classified as maturing. Each slide (liver and gonad tissue) was examined completely for presence or absence of gross lesions or other abnormalities followed by semi-quantification of macrophage aggregates (MA) in gonad and liver tissue and eosinophils and lymphocytes in hepatic tissue in a randomly chosen field of view (10x). An index for semi-quantification was formulated as follows for the fish captured in the fisheries: 0 = no MA or lymphocytes, 1 = 1-25% of the tissue contained MA or lymphocytes, 2 = 26-50% of the tissue contained MA or lymphocytes,
3 = 51-75% of the tissue contained MA or lymphocytes, 4 = 75-100% of the tissue contained MA or lymphocytes.

Contaminant analysis

A sub-sample of livers (n=97) and gonads (n=98) were analyzed for 18 chlorinated pesticides and 28 PCB congeners (Table 1). This represented 11-17 males and 10-14 females from each sampling location.

Extraction and cleanup procedures of sturgeon tissues were based on the methods described by Price et al. (1986) and Gundersen et al. (1998). Liver and gonad samples were homogenized using a Brinkman Polytron® tissue homogenizer and a portion was removed for measurement of moisture content. Subsamples of tissue homogenates (~ 5 g) were combined with sodium sulfate (~ 50 g) and ground to a fine powder using a mortar and pestle. Dried tissues were Soxhlet extracted (10 h) with 170 ml of 1:1 petroleum ether/hexane (v/v spectral grade; Sigma-Aldrich, St. Louis, MO, USA). Extracts were concentrated to less than 15 ml with a rotary evaporator and transferred to tared vials, where the remaining solvent was evaporated to dryness using a warm water bath and a stream of pure nitrogen (N_2). The amount of lipid in each sample

was determined gravimetrically. Lipid extracts were cleaned using 20 g florisil-packed glass columns (400 X 19 mm), and PCBs and chlorinated pesticides were eluted with 6% ethyl ether/petroleum ether (v/v). PCBs and pesticides were fractionated into two eluates using 5 g silica gel-packed glass columns (10.5 X 300 mm). The first fraction (PCBs and p,p'-DDE) was eluted with hexane. The second fraction (chlorinated pesticides) was eluted with benzene.

The cleaned fractions were analyzed using a Varian CP-3800 gas chromatograph equipped with a ^{63}Ni electron capture detector, a CP-8200 AutoSampler, a Star Chromatography Workstation (version 5) and a SPB-608 fused silica capillary column (30 mm X 0.25 mm X 0.25 µm film thickness, Supelco, Bellefonte, PA). Gas chromatographic parameters used were: carrier gas helium (1.5 ml/min), makeup gas nitrogen, detector temperature 300 ºC, injector temperature 290 ºC and oven temperature 150 ºC (4 min) to 290 ºC (10 min) at 8 ºC/min. Organochlorine pesticides were quantified from individually resolved peak areas with corresponding peak areas of external standards (Supelco). Individual PCB congeners purchased from AccuStandard (New Haven, CT, USA) were used to make external standards containing the 28 selected PCB congeners.

Quality assurance measures included the analysis of reagent blanks, duplicates and matrix spike samples. Percent recoveries of PCB congeners and organochlorine pesticides in matrix spikes were between 90 and 110%; therefore, sample extracts were not corrected for percent recovery. Detection limits for individual PCB congeners and chlorinated pesticides were 0.01 µg/g wet weight. The State of Oregon Environmental Quality Laboratories and Applied Research, Organic Laboratory section analyzed two tissue homogenates for chlorinated pesticides (interlaboratory comparison). The relative percent difference of organochlorine pesticide

concentrations reported by the two laboratories in the two samples differed by an average of less than 17%.

Aging of Fish

Ages of fish sampled in 2001 were determined by pectoral fin spine analysis following the procedures described in Beamesderfer et al. (1989). Two independent determinations were conducted at The Oregon Department of Fish and Wildlife and at University of California-Davis. The percentage of fish with identical age assignments was 27%. The percentage of fish aged within 1 year by the different readers was 45%, within 2 years was 22%, within 3 years was 2%, and > 5 years was 4%. Ages of fish that were not in agreement between the two determinations were averaged.

Western Blot Analysis

Hepatic microsomes were prepared by differential centrifugation according to Carpenter et al. (1990) and stored at –80^{o}C until use. Briefly, livers were minced in ice-cold buffer (0.1 M Tris-acetate, pH 7.4; 0.1 M KCl; 1 mM EDTA; 20 μM butylated hydroxytoluene; and 1 mM phenylmethylsulfonylfluoride) and homogenized in 4 volumes of the same buffer. The homogenate was centrifuged at 10,000 g for 30 min and the resulting supernatant was centrifuged at 100,000 g for 90 min. The microsomal pellet was resuspended in buffer (0.1 M phosphate buffer, pH 7.25; 20% glycerol; and 1 mM EDTA). Microsomes were stored at –80^{o}C until use. The putative white sturgeon hepatic cytochrome P450 3A was measured in microsomes by western blotting using a polyclonal antibody generated against rainbow trout LMC5 (3A27). Microsomal CYP3A protein was measured using Western immunoblot techniques according to

Towbin et al. (1979) with modifications. Briefly, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed using 8% polyacrylamide precast minigels. Membranes were prepared according to the manufacturers recommendation and proteins were transferred to membranes followed by incubation with rabbit anti-trout antibody (a generous gift from Dr. D. Buhler). Membranes were rinsed with PBS-Tween and incubated with horseradish peroxidase conjugated secondary antibodies (anti-rabbit) for detection of oxidized luminol (Amersham Biosciences, Piscataway, NJ). The chemiluminescent signal was captured on film (Hyperfilm ECL, Amersham Biosciences, Piscataway, NJ). Films were scanned for quantification.

Statistics

All mean comparisons between physiological parameters, tissue contaminant load, river location and sex of fish were conducted using a one-way ANOVA with a Bonferroni procedure. All correlations between tissue contaminant load and physiological parameters were conducted using reciprocal-Y regression. All analyses were performed using Statview® software and the accepted level of significance for all tests was $p < 0.05$.

Results

All 18 of the chlorinated pesticides examined in tissues from wild fish were detected in at least some of the samples (Table 2). Metabolites of p,p'-DDT (p,p'-DDE and p,p'-DDD) were consistently found in fish at relatively high levels. Concentrations of DDT and its metabolites were always DDE > DDD and DDT in both livers and gonads (Figure 2). There were no

differences in toxicant levels between tissues. Of the 28 PCB congeners examined, 26 were detected in at least some of the samples (Table 3).

Total DDT (DDD + DDE + DDT), total pesticides (all pesticides detected – total DDT) and PCBs (total of all detected) were significantly higher in livers and gonads of fish from Bonneville reservoir compared to other locations (Figure 3). Fish from the Bonneville reservoir had significantly lower TAG plasma concentrations and GSI than two of the other locations (Figure 4). Fish from Bonneville also had significantly lower Ca plasma concentrations and CF compared to all other locations.

A negative correlation was seen between plasma TAG and total DDT, pesticides and PCBs in livers (Table 4). To varying degrees, this was also true for TAG compared to contaminants in gonads and for CF compared to contaminants in livers and gonads. Although significant relationships were observed, r^2 values indicated that a large amount of variation was present within the data.

Plasma concentrations of T were higher in males than females at all sample locations except Bonneville (Figure 5). Males from the estuary had significantly higher levels of KT than females, but this was not seen at other locations. Males from the estuary had significantly higher plasma T and KT than males in Bonneville and John Day reservoirs. Plasma concentrations of E2 were very low in all fish examined (Table 5). No differences were observed between either sex or location.

Plasma Vtg was at or very near the detection limit of the assay for all fish sampled in the estuary and Bonneville (Figure 5). Some males and immature females from The Dalles and John Day reservoirs had detectable levels of Vtg. Males from John Day had significantly higher concentrations of Vtg than all other locations. Females from the Dalles had concentrations of Vtg

that were nearly significant when compared to females from the estuary ($p = 0.060$) as well as when compared to females from Bonneville ($p = 0.058$). There was no correlation between plasma Vtg and any of the pesticides or PCBs that were monitored.

Gonadal histology revealed a total of 82 females, 73 males and 3 hermaphrodites from the two years of sampling. Sixteen gonad samples contained only adipose tissue and no gonial cells. Of the females, 81 were immature (all Stage 2 except for three Stage 1 females) and one was a maturing female (Stage 3; early vitellogenesis). Of the males, 66 were immature (all Stage 2), one male was in Stage 3 of gonadal development (onset of meiosis) and 6 males were in Stage 5 of development (spermiation). No maturing fish were captured in Bonneville Reservoir. All of the maturing males had significantly higher levels of plasma androgens ($T = 92.2 \pm 20.9$, $KT = 84.0 \pm 16.4$ ng/ml) than immature males ($T = 5.1 \pm 1.1$, $KT = 4.3 \pm 1.0$ ng/ml). All three of the hermaphroditic fish had predominately female ovotestes. Two of the three fish were captured in Bonneville Reservoir and the other was from the estuary. Several fish showed irregular ovarian plasma membranes and intrusion of muscle into the ovary. Macrophage aggregates were found in both female and male gonadal tissue and were most often found to contain melanin.

Liver histology revealed a high incidence of MA and lymphocytes. However, no pattern was discernible with regard to contaminant level. Eleven liver samples had a very high incidence of MA and/or lymphocytes; seven of these fish were from the Bonneville reservoir, two were from the estuary and one each were from The Dalles and John Day reservoirs.

A negative correlation was seen between plasma T and total DDT, pesticides and PCBs in livers of male white sturgeon (Figure 6). These relationships were also seen for contaminants in gonads (Figure 7). To varying degrees, this was also true for plasma KT and GSI when compared to contaminants in gonads and livers (Table 6).

Spermatogonia proliferation (Stage 2) in white sturgeon is associated with increased circulating androgen concentrations regardless of age or size (Feist et al. 2004). In immature wild white sturgeon, T concentrations greater than 4 ng/ml may be used to differentiate Stage 2 males from Stage 1 males and immature females (Webb et al. 2002). All 66 immature males in our study were in Stage 2 of gonadal development, yet 47 (71.2%) had plasma T concentrations that were less than 4 ng/ml. Of the 48 Stage 2 males that were analyzed for toxicants, 31 had levels of T that were less than 4 ng/ml. In addition, no males with liver contaminant levels above 9.5 ppm (total DDT), 5.6 ppm (total pesticides) or 2.8 ppm (PCBs) had plasma T concentrations greater than 4 ng/ml (figure 6). Concentrations of toxicants in gonads where this was observed for total DDT, total pesticides and PCBs occurred at 11.6, 3.7 and 2.5 ppm, respectively (figure 7).

Aging of fish by pectoral fin spine analysis in 2001 revealed that sturgeon from Bonneville (18.3 ± 1.0 yrs, range = 14-27) and John Day (17.4 ± 0.4 yrs, range = 14-20) were significantly older than those sampled in The Dalles (14.8 ± 0.5 yrs, range = 10-19). Bonneville fish were also significantly older than estuary fish (14.6 ± 1.0 yrs, range = 10-17).

To investigate the possibility that DDE reduces plasma androgens by increasing steroid metabolism and excretion via up-regulation of liver cytochrome P450 isozymes, we conducted a preliminary and purely quantitative western blot analysis to measure the putative cytochrome P450 3A in microsomes. This enzyme, in trout, is responsible for hydroxylating steroids as a first step for metabolism and excretion (see Lee et al. 2001). A western blot for this isozyme is shown in Figure 8. Male sturgeon with higher liver content of DDE showed increased immunoreactivity for P450 3A.

Discussion

The life history of white sturgeon may make them particularly susceptible to the actions of persistent bioaccumulating pollutants. These fish are bottom dwellers and feed on benthic prey items that are closely associated with sediments containing hydrophobic pollutants. Sturgeon can live for over 100 years and females mature between 16 and 35 years of age (DeVore et al. 1995). Thus, toxicants may accumulate and have deleterious effects over a long period of time before the fish reach a stage when they are capable of reproduction. A recent study in the Columbia River found that sturgeon contained the highest body burdens of contaminants out of 12 species of fish examined (EPA 2002). Levels of toxicants seen in the present study were comparable to those found by EPA and also to levels previously reported by our laboratory (Foster et al. 2001a, 2001b).

Fish trapped behind the oldest of the dams examined (Bonneville) had the highest contaminant loads and the lowest condition factor, gonad size, and plasma androgens and triglycerides. These fish also had the highest incidence of gonadal abnormalities. This suggests that endocrine disrupting chemicals (EDCs) may be accumulating behind dams over time.

It has recently been determined that past operation of the dam at Bonneville has resulted in areas within the reservoir that have very high levels of PCBs (URS Corp. 2002). In our study, Bonneville fish were older than fish from two of the other sampling locations. Fish from this reservoir also grow slower and females mature at a later age than other locations (Beamesderfer et al. 1995). Thus, these fish may be exposed to higher levels of contaminants and for longer periods of time than comparably sized fish from other areas of the river. Food availability may be the main cause for reduced growth in Bonneville fish, but effects of toxicants cannot be ruled

out. The negative correlations found between plasma triglycerides and condition factor with tissue burdens of pesticides and PCBs add strength to this possibility.

Our laboratory has previously documented a negative correlation between plasma androgens and tissue content of p,p'-DDE for Columbia River sturgeon (Foster et al. 2001b). In the present study, negative correlations were observed between both plasma androgens and GSI of males when compared to total DDT, total pesticides and PCBs. Our sample size for this study was much greater than our previous research which may explain why these relationships were not seen in the earlier study. p,p'-DDE has also been shown to have demasculinizing effects in the guppy (*Poecilia reticulata*) (Baatrup and Junge 2001; Bayley et al. 2002). Our data also suggest that DDT and its metabolites may reach threshold levels in liver and gonad, above which, the fish are incapable of elevating plasma T concentrations. This may result in the inability of males with high body burdens of contaminants to attain sexual maturity.

We have preliminary evidence that the mechanism of action of plasma androgen reduction by p,p'-DDE, or possibly by other pesticides or PCBs, is by increasing steroid metabolism through up-regulation of P450 3A. DDE has been shown to induce this isozyme and increase metabolism of testosterone in mice (Dai et al. 2001). Rainbow trout *(Oncorhynchus mykiss*) injected with DDE, however, showed a decrease in P450 3A-dependent 6 beta-hydroxylation of testosterone (Machala et al. 1998). The dose used for the rainbow trout study was much higher (50 mg/kg) than levels seen in wild fish in our study and may not have simulated the effects of chronic exposure to lower concentrations of DDE.

Our finding that plasma androgens were higher in males than females (except in the Bonneville reservoir) has been documented by our laboratory previously. We have used differences in plasma steroids between males and females to develop a model for sexing both

immature and maturing wild white sturgeon and for determining sex of cultured fish at an early age (Feist et al. 2004; Webb et al. 2002).

Although banned for use in the United States in 1973, DDT and its metabolites are still being detected in sturgeon at relatively high levels. This indicates that this compound is extremely persistent in the environment. Tissue burdens were always DDE > DDD and DDT indicating that aerobic degradation of DDT (primarily yielding DDE) is the main metabolic pathway as opposed to anaerobic degradation (primarily yielding DDD) (see Spencer et al. 1996). This suggests that the most likely source of DDT metabolites is from agricultural runoff of the parent compound as opposed to anaerobic degradation of DDT in sediments.

The type and source of xenoestrogen(s) responsible for elevating plasma Vtg in males and immature females from The Dalles and John Day reservoirs remains uncertain. None of the pesticides or PCBs monitored in this study were correlated with plasma Vtg. Fish exposed in our laboratory to the pesticides (permethrin and pyriproxyfen) or herbicides (atrazine and simazine), which are currently being used in agricultural practices in the Columbia basin, did not show increases in plasma Vtg (data not shown). Caged sturgeon, in areas of the river where some wild fish had elevated Vtg, also did not show an increase in this protein (data not shown). This suggests that wild fish are either being exposed to potential EDCs for longer periods of time or are bioaccumulating them through ingestion of prey.

Other candidates for induction of Vtg include the alkyphenols which have been shown to be weakly estrogenic in fish (Jobling et al. 1996; White et al. 1994). Fish exposed to octylphenol and nonylphenol in our laboratory experienced increased plasma Vtg (data not shown), but we are unable to find a likely source for alkylphenolic compounds in The Dalles and John Day reservoirs. There are many sources of alkylphenols in the estuary and Bonneville reservoir, yet

we found no elevated Vtg in wild sturgeon sampled in this area of the river. The cause of elevated Vtg in wild fish is most likely due to other EDCs or metabolites of toxicants not yet identified, or combinations of compounds.

The overall results of this study indicate that exposure to environmental contaminants may be affecting both growth and reproductive physiology of sturgeon in some areas of the Columbia River. Questions remain, however, as to what effects these contaminants have on the ability of sturgeon to successfully reproduce. It is unknown if lowered energy reserves, GSI and androgens and elevated Vtg actually inhibit or decrease the ability of sturgeon to mature and spawn. Due to the slot-size limit (fish that are 110-137 cm in fork length), the majority of wild fish sampled in this study were immature. Larger sturgeon, that have reached a sufficient size and age to mature, must be examined to determine possible deleterious effects of contaminants on reproduction. Different year classes of sturgeon also need to be investigated to determine if toxicants are bioaccumulating as the fish age. Finally, prey items need to be examined for the presence of EDCs to determine if sturgeon are acquiring these compounds from their diet or other sources.

The poor reproductive success of sturgeon in impounded areas of the Columbia River is most likely due to a wide variety of stressors including food availability, poor spawning habitat and changes in flow and temperature. Exposure to environmental contaminants may be an additional stressor that is contributing to this reduced reproductive fitness.

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Table 1. Chlorinated pesticides and PCBs measured in Columbia River white sturgeon livers and gonads.

Chlorinated Pesticide	PCB (IUPAC)
Aldrin	2,2',5-Trichlorobiphenyl (18)
α-bhc	2,4,4'-Trichlorobiphenyl (28)
β-bhc	2,2',3,5'-Tetrachlorobiphenyl (44)
γ-bhc	2,2',5,5'-Tetrachlorobiphenyl (52)
δ-bhc	2,3,4,4'-Tetrachlorobiphenyl (60)
p,p'-DDD	2,3',4,4'-Tetrachlorobiphenyl (66)
p,p'-DDE	2,4,4',5-Tetrachlorobiphenyl (74)
p,p'-DDT	3,3',4,4'-Tetrachlorobiphenyl (77)
Dieldrin	2,2',3,4,5'-Pentachlorobiphenyl (87)
Endrin	2,2',4,4',5-Pentachlorobiphenyl (99)
Endrin aldehyde	2,2',4,5,5'-Pentachlorobiphenyl (101)
Endrine ketone	2,3,3',4,4'-Pentachlorobiphenyl (105)
Endosulfan I	2,3,3',4',6-Pentachlorobiphenyl (110)
Endosulfan II	2,3',4,4',5-Pentachlorobiphenyl (118)
Endosulfan sulfate	3,3',4,4',5-Pentachlorobiphenyl (126)
Heptachlor	2,2',3,3',4,4'-Hexachlorobiphenyl (128)
Heptachlor epoxide	2,2',3,4,4',5'-Hexachlorobiphenyl (138)
p,p'-methoxychlor	2,2',3,5,5',6-Hexachlorobiphenyl (151)
	2,2',4,4',5,5'-Hexachlorobiphenyl (153)
	2,3,3',4,4',5-Hexachlorobiphenyl (156)
	3,3',4,4',5,5'-Hexachlorobiphenyl (169)
	2,2',3,3',4,4',5-Heptachlorobiphenyl (170)
	2,2',3,4,4',5,5'-Heptachlorobiphenyl (180)
	2,2',3,4,4',5',6-Heptachlorobiphenyl (183)
	2,2',3,4',5,5',6-Heptachlorobiphenyl (187)
	2,2',3,3',4,4',5,5'-Octachlorobiphenyl (194)
	2,2',3,3',4,5,5',6'-Octachlorobiphenyl (199)
	2,2',3,4,4',5,5',6-Octachlorobiphenyl (203)

Table 2. Average concentration ± SE of chlorinated pesticides (mg/kg wet weight; lipid normalized) in livers (n=97) and gonads (n=98) of white sturgeon from the Columbia River.

	Livers		Gonads	
Pesticide	(D)	mg/kg	(D)	mg/kg
Aldrin	2	0.002 ± 0.002	5	0.011 ± 0.006
α-bhc	19	0.039 ± 0.009	26	0.023 ± 0.005
β-bhc	14	0.115 ± 0.046	11	0.023 ± 0.005
γ-bhc	8	0.024 ± 0.011	21	0.047 ± 0.014
δ-bhc	9	0.019 ± 0.007	15	0.154 ± 0.127
p,p'-DDD	86	1.863 ± 0.544	93	1.619 ± 0.400
p,p'-DDE	97	18.40 ± 7.313	98	10.60 ± 2.086
p,p'-DDT	28	0.274 ± 0.103	41	0.259 ± 0.073
Dieldrin	16	0.134 ± 0.045	15	0.031 ± 0.009
Endrin	10	0.114 ± 0.060	11	0.022 ± 0.007
Endrin aldehyde	16	0.108 ± 0.062	13	0.064 ± 0.032
Endrine ketone	8	0.038 ± 0.165	2	0.010 ± 0.007
Endosulfan I	34	0.161 ± 0.044	45	0.133 ± 0.025
Endosulfan II	9	0.108 ± 0.051	14	0.087 ± 0.047
Endosulfan sulfate	3	0.005 ± 0.003	8	0.008 ± 0.003
Heptachlor	8	0.018 ± 0.008	13	0.037 ± 0.019
Heptachlor epoxide	15	0.081 ± 0.031	25	0.074 ± 0.024
p,p'-methoxychlor	14	0.112 ± 0.044	5	0.027 ± 0.017

(D) = number of detections

Table 3. Average concentration ± SE of PCBs (mg/kg wet weight; lipid normalized) in livers (n=97) and gonads (n=98) of white sturgeon from the Columbia River.

	Livers		Gonads	
PCB	(D)	mg/kg	(D)	mg/kg
28	3	0.020 ± 0.011	0	
44	6	0.055 ± 0.042	4	0.004 ± 0.002
52	3	0.038 ± 0.024	3	0.024 ± 0.105
60	19	0.125 ± 0.033	11	0.163 ± 0.129
66	8	0.131 ± 0.066	2	0.025 ± 0.020
74	2	0.008 ± 0.006	4	0.037 ± 0.022
87	1	0.006 ± 0.006	2	0.008 ± 0.006
99	12	0.101 ± 0.036	12	0.077 ± 0.041
101	28	0.238 ± 0.088	24	0.217 ± 0.131
105	14	0.135 ± 0.051	9	0.033 ± 0.016
110/77	12	0.060 ± 0.019	17	0.128 ± 0.050
118	9	0.054 ± 0.020	10	0.152 ± 0.085
126	6	0.035 ± 0.016	5	0.024 ± 0.018
128	1	0.007 ± 0.007	6	0.043 ± 0.031
138	28	0.258 ± 0.071	28	0.233 ± 0.072
151	4	0.025 ± 0.015	7	0.032 ± 0.014
153	18	0.264 ± 0.101	20	0.157 ± 0.062
156	6	0.035 ± 0.018	7	0.013 ± 0.006
169	2	0.007 ± 0.005	0	
170	3	0.006 ± 0.003	3	0.003 ± 0.001
180	3	0.030 ± 0.026	3	0.001 ± 0.001
183	9	0.042 ± 0.015	13	0.029 ± 0.010
187	20	0.163 ± 0.047	21	0.113 ± 0.032
194	4	0.018 ± 0.009	1	0.001 ± 0.001
199	10	0.022 ± 0.007	10	0.065 ± 0.030
203/170	10	0.043 ± 0.017	10	0.016 ± 0.008

(D) = number of detections

Table 4. Regression analyses of TAG and CF versus various contaminants in livers and gonads of Columbia River white sturgeon.

	LIVERS				GONADS			
	TAG		CF		TAG		CF	
Contaminant	r^2	p	r^2	p	r^2	p	r^2	p
Total DDT	0.60	<0.001	0.08	<0.005	0.20	<0.001	0.11	<0.001
Total Pesticides	0.48	<0.001	0.15	<0.001	0.04	<0.050	0.18	<0.001
Total PCBs	0.60	<0.001	0.11	<0.002	0.10	<0.002	0.07	<0.008

Table 5. Average concentration ± SE of plasma 17β-estradiol (ng/ml) in male (n=19-24) and female (n=21-23) white sturgeon at four locations from the Columbia River.

	LOCATION			
	Estuary	Bonneville	The Dalles	John Day
Females	0.09 ± 0.02	0.11 ± 0.03	0.13 ± 0.02	0.28 ± 0.05
Males	0.16 ± 0.03	0.07 ± 0.01	0.14 ± 0.03	0.38 ± 0.10

Table 6. Regression analyses of KT and GSI versus various contaminants in livers and gonads of male Columbia River white sturgeon.

	LIVERS				GONADS			
	KT		GSI		KT		GSI	
Contaminant	r^2	p	r^2	p	r^2	p	r^2	p
Total DDT	0.08	<0.050	0.24	<0.001	0.11	<0.020	0.21	<0.001
Total Pesticides	NS	NS	0.15	<0.006	NS	NS	0.22	<0.001
Total PCBs	0.16	<0.004	NS	NS	NS	NS	0.10	<0.030

NS denotes “not significant”

Figure Legends.

Figure 1. Sample sites for white sturgeon from the Columbia River in the estuary near Astoria, OR (EST) and the reservoirs behind Bonneville (B), The Dalles (TD) and John Day (JD) dams.

Figure 2. Mean concentrations ± SE of DDT and its metabolites in livers (n=97) and gonads (n=98) of white sturgeon from all sample areas combined. Means with different letters indicate a significant difference within a tissue for an ANOVA with $p<0.05$.

Figure 3. Mean concentrations ± SE of total DDT (DDD + DDE + DDT), total pesticides (all pesticides detected - total DDT) and PCBs (total of all detected) in livers and gonads of white sturgeon from 4 locations on the Columbia River. Each bar represents a sample size of 22-28. All concentrations are lipid normalized. * denotes statistically different from other locations for an ANOVA with $p<0.05$.

Figure 4. Mean plasma concentrations ± SE of triacylglycerides (TAG), condition factor (CF), calcium (Ca) and gonadosomatic index (GSI) in white sturgeon from 4 locations on the Columbia River. Each bar represents a sample size of 42-45. Means with different letters indicate a significant difference between locations for an ANOVA with $p<0.05$.

Figure 5. Mean plasma concentrations ± SE of testosterone (T), 11-ketotestosterone (KT) and vitellogenin (Vtg) in male and immature female white sturgeon from 4 locations on the Columbia River. Each bar represents a sample size of 19-24. Means with different letters or numbers indicate a significant difference between locations or between sexes within a location, respectively, for an ANOVA with $p<0.05$. Lower right panel shows individual Vtg concentrations.

Figure 6. Individual plasma testosterone (T) versus total DDT, total pesticides or total PCB concentrations in livers of male white sturgeon. Reciprocal-Y regression: $p< 0.001$ and $r^2 =0.79$ for DDT, $p< 0.001$ and $r^2 =0.56$ for pesticides and $p< 0.001$ and $r^2 =0.80$ for PCBs. All males with toxicant levels higher than those denoted by the vertical dashed line have less than 4 ng/ml of T.

Figure 7. Individual plasma testosterone (T) versus total DDT, total pesticides, or total PCB concentrations in gonads of male white sturgeon. Reciprocal-Y regression: $p< 0.001$ and $r^2 =0.85$ for DDT, $p< 0.001$ and $r^2 =0.31$ for pesticides and $p< 0.001$ and $r^2 =0.82$ for PCBs. All males with toxicant levels higher than those denoted by the vertical dashed line have less than 4 ng/ml of T.

Figure 8. Western blot of P450 3A protein in individual livers of male white sturgeon with varying levels of liver DDE.

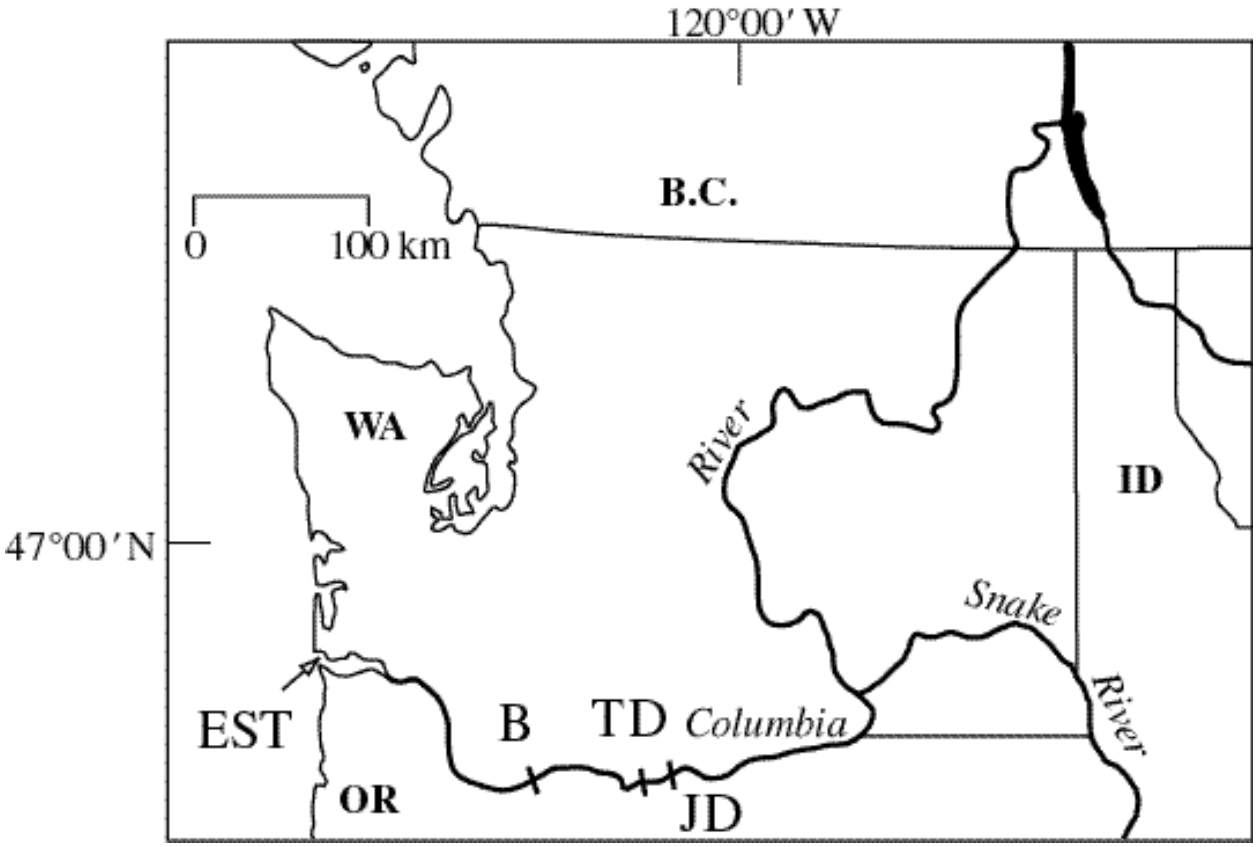
120°00′ W
B.C.
0
100 km
WA
River
ID
47°00′N
Snake
River
EST
B
TD
Columbia
OR
JD

Figure 1.

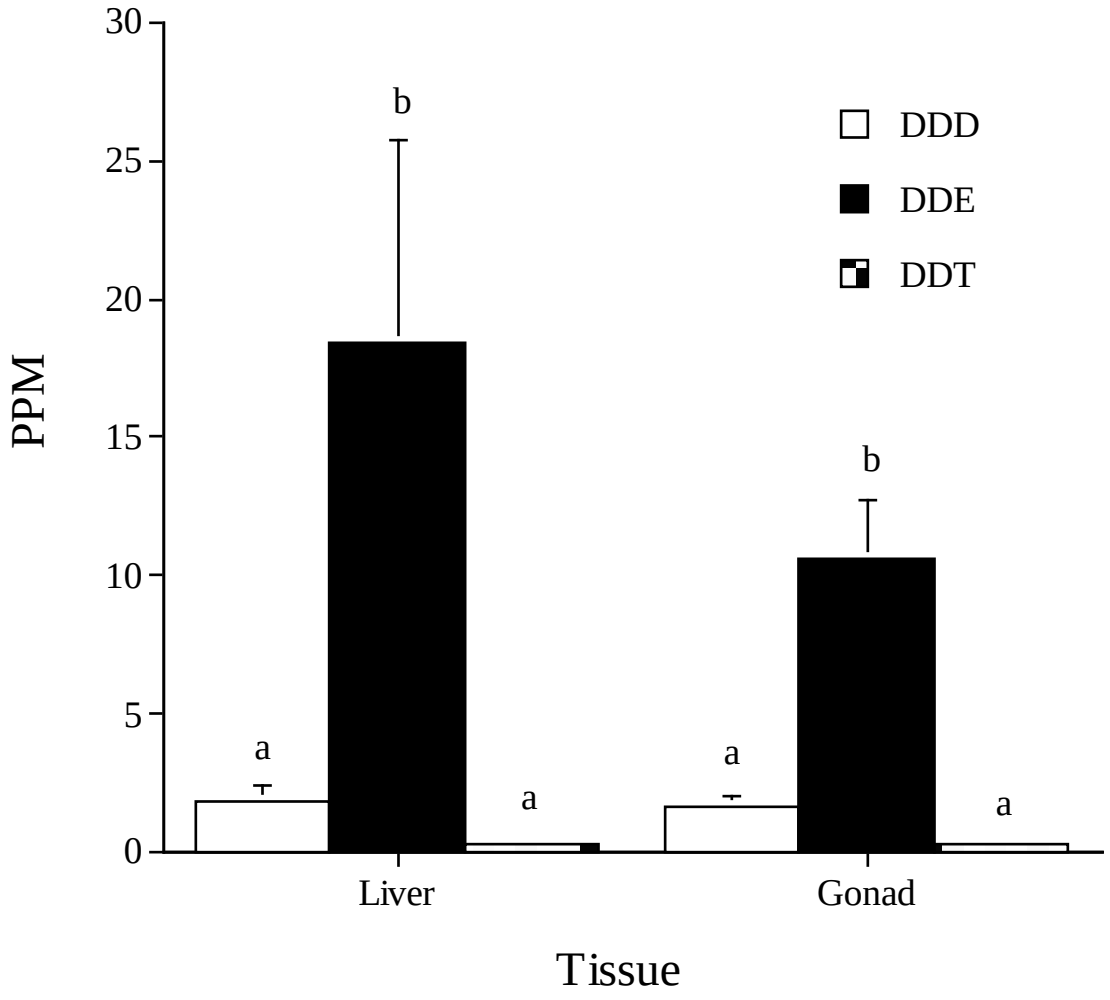

Figure 2.

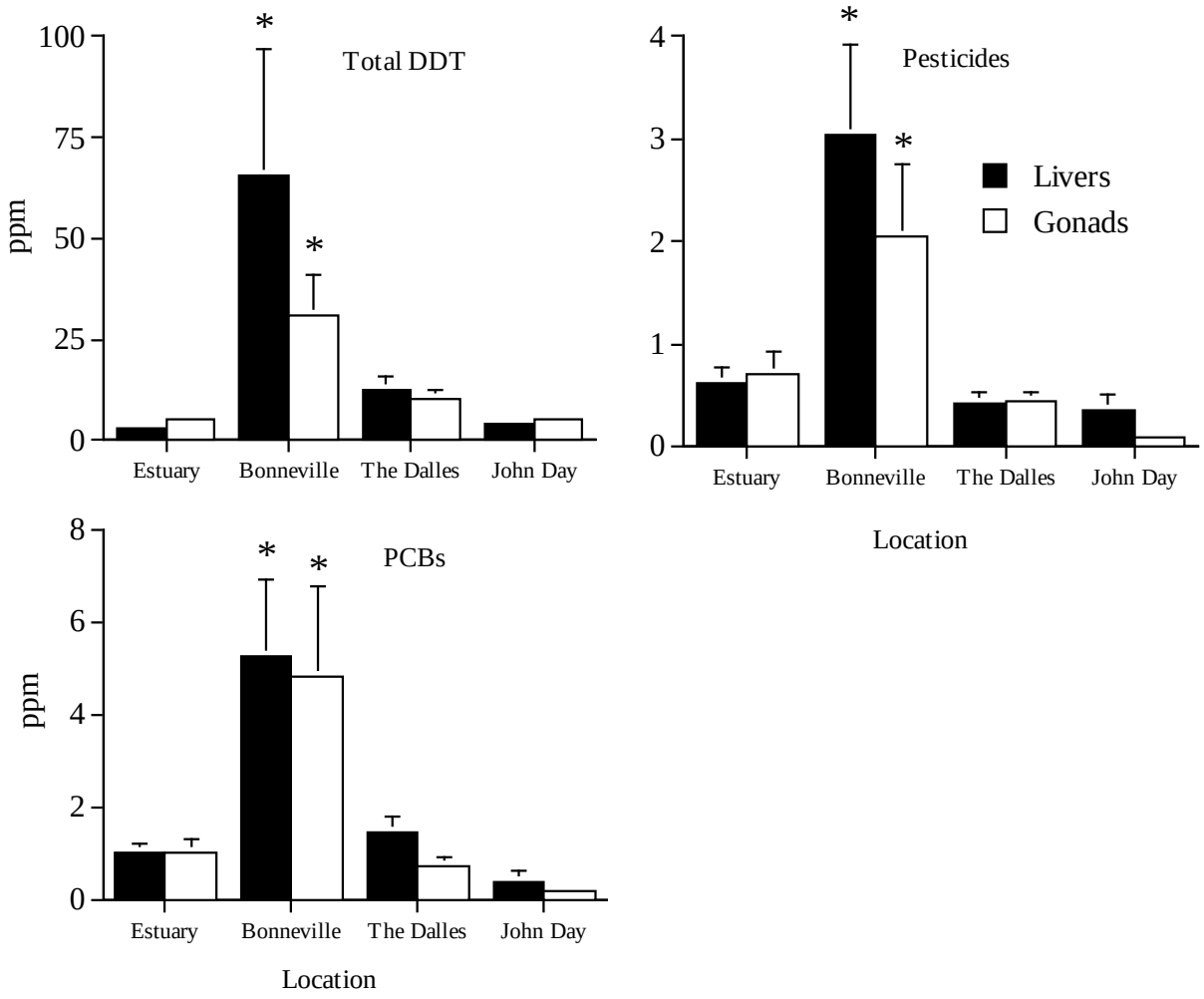
Total DDT
ppm
100
75
50
25
0
Estuary
Bonneville
The Dalles
John Day
Pesticides
4
3
2
1
0
Livers
Gonads
Location
PCBs
8
6
4
2
0
Location

Figure 3.

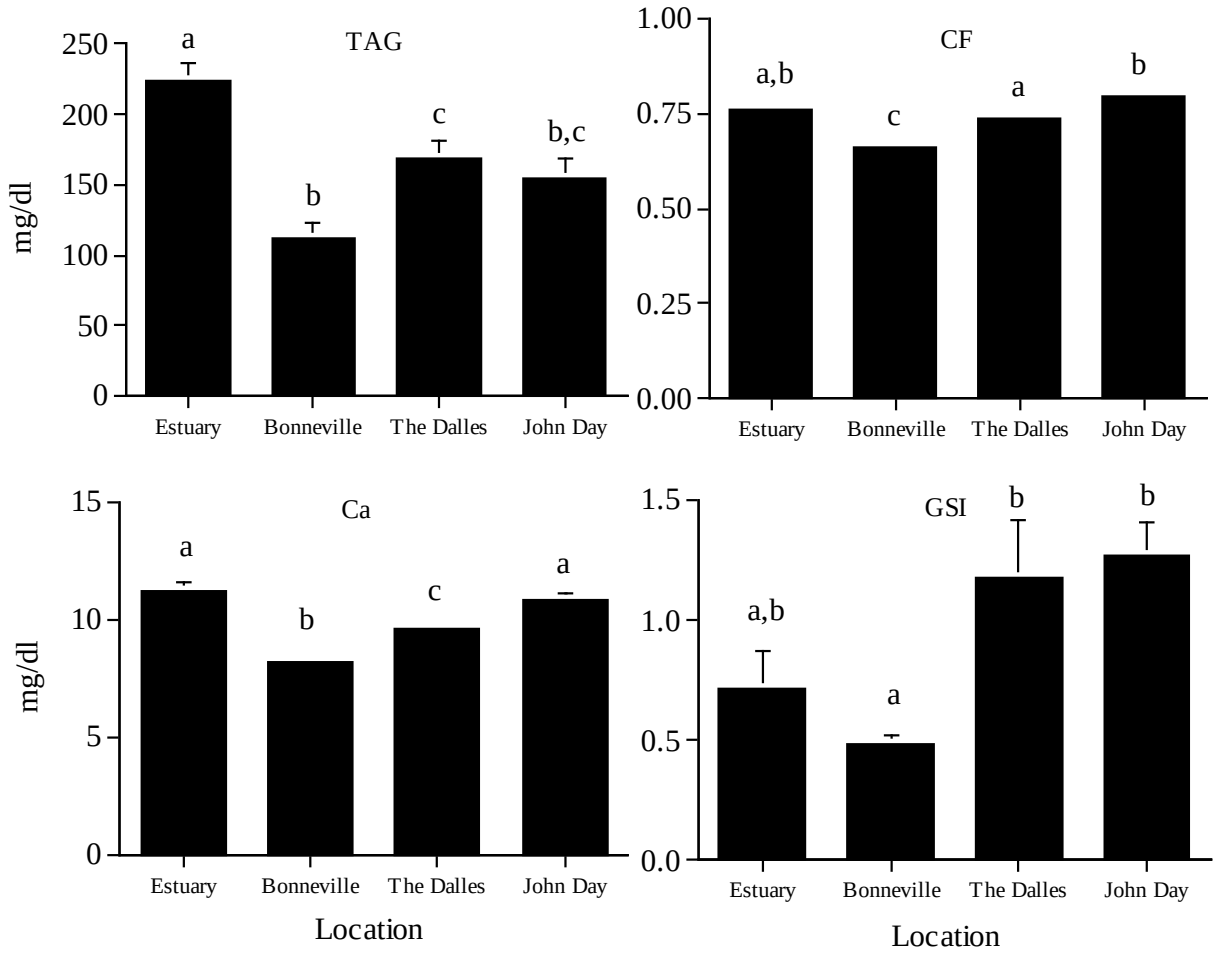
TAG
mg/dl
250
200
150
100
50
0
a
b
c
b,c
Estuary
Bonneville
The Dalles
John Day
CF
1.00
0.75
0.50
0.25
0.00
a,b
c
a
b
Ca
15
10
5
0
a
b
c
a
Location
GSI
1.5
1.0
0.5
0.0
a,b
a
b
b
Location

Figure 4.

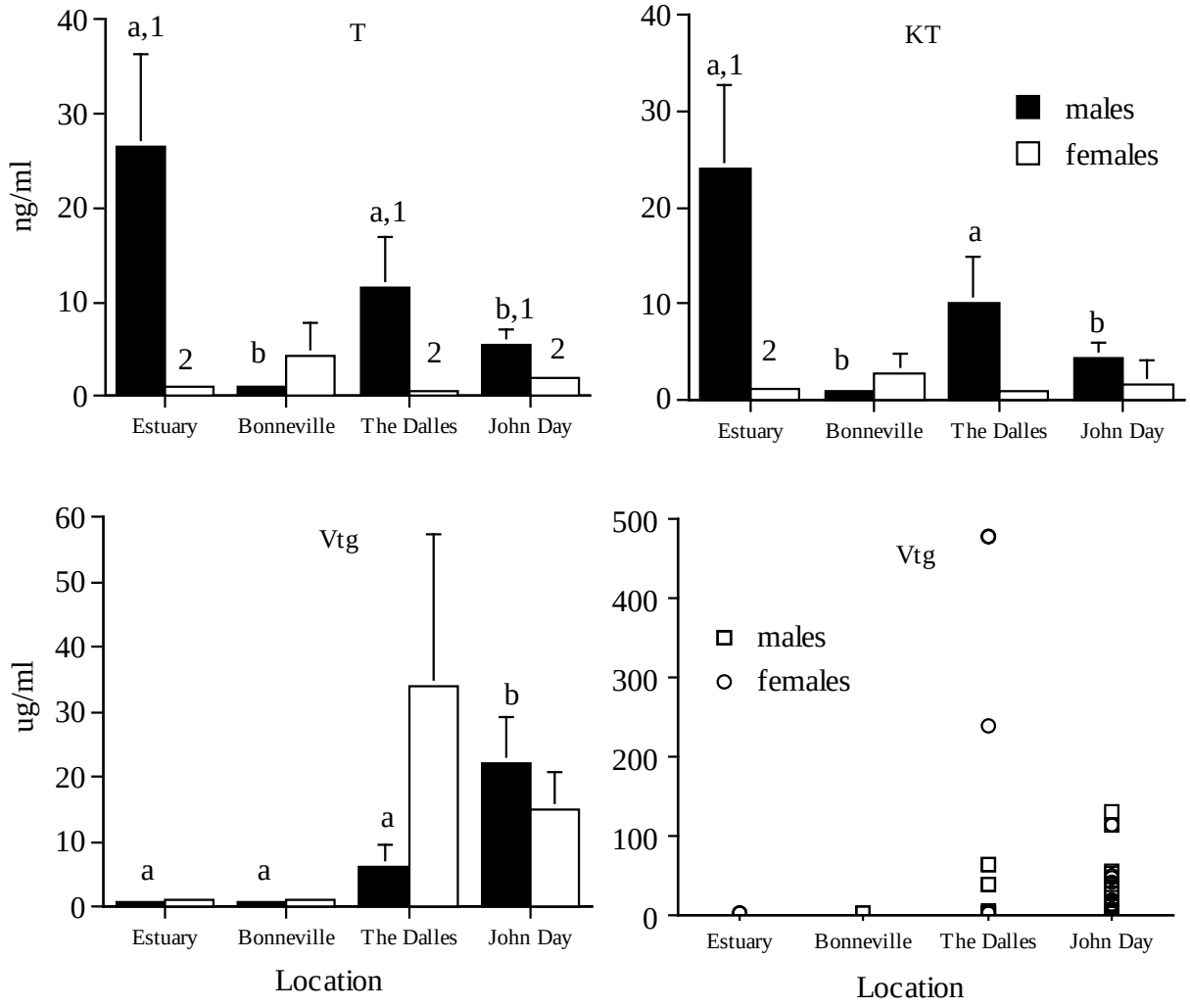
T
KT
Vtg
Vtg
ng/ml
ug/ml
males
females
a,1
b
a
b,1
2
Estuary
Bonneville
The Dalles
John Day
Location

Figure 5.

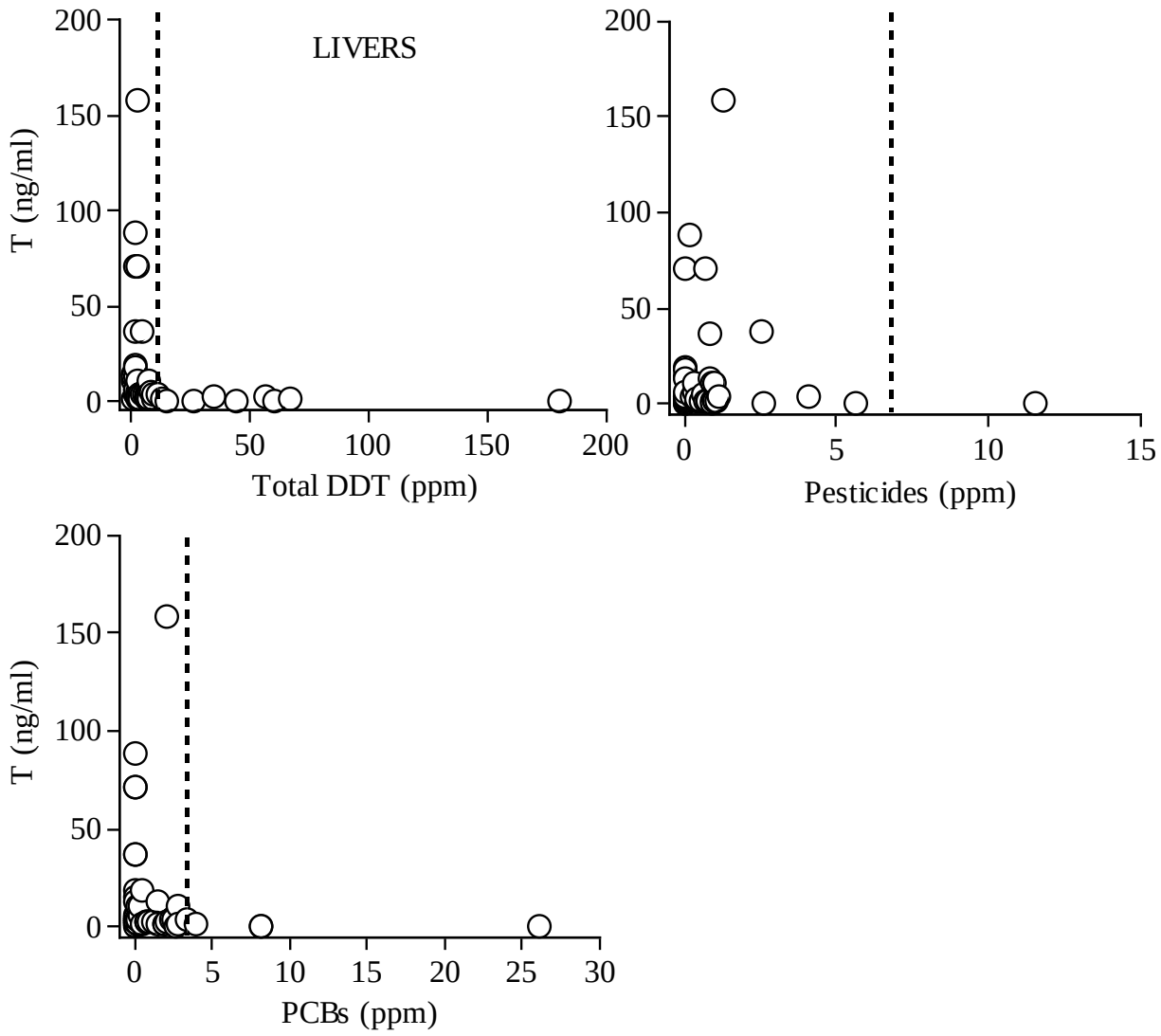
LIVERS
T (ng/ml)
Total DDT (ppm)
Pesticides (ppm)
PCBs (ppm)
0
50
100
150
200
5
10
15
20
25
30

Figure 6.

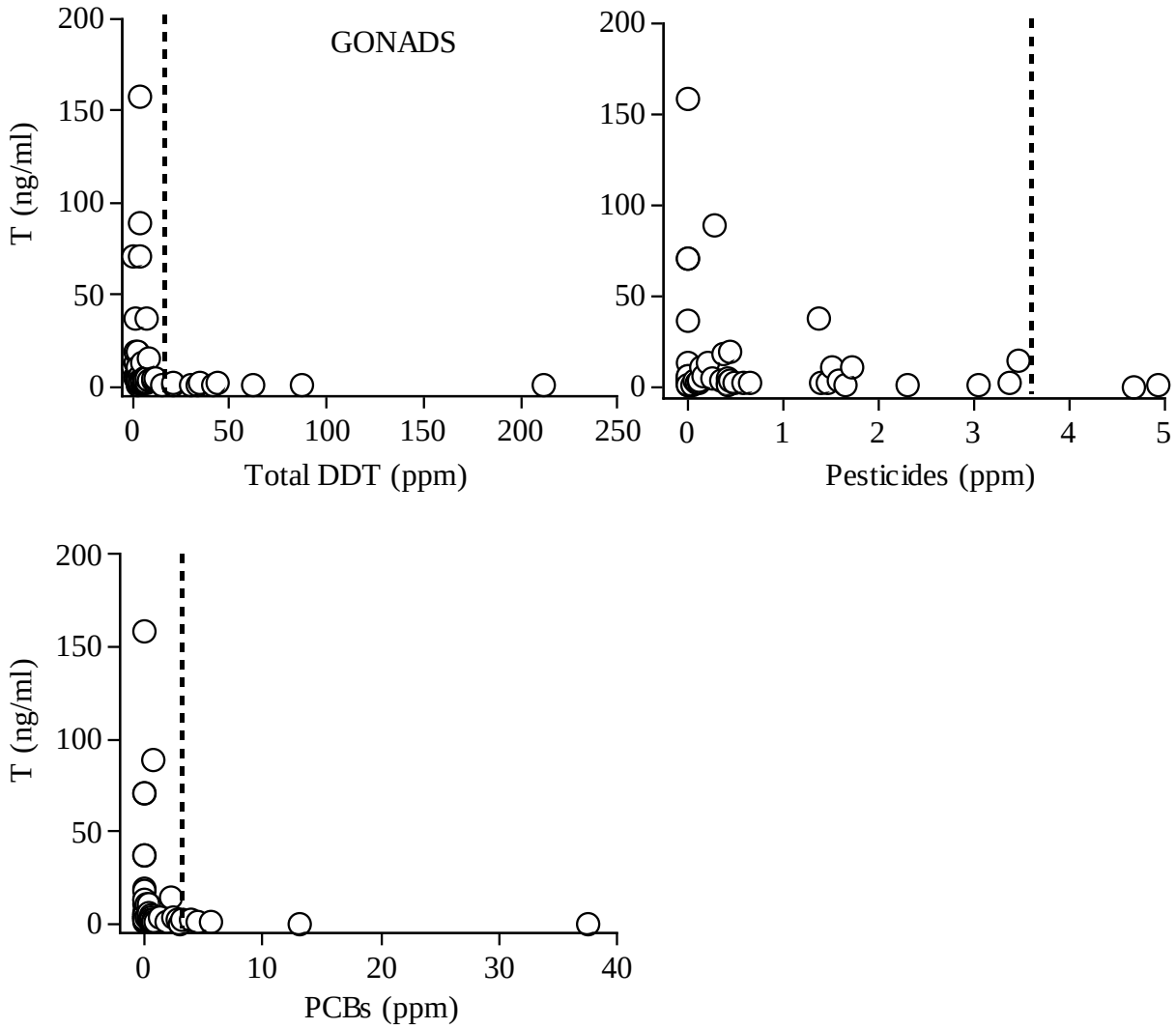
GONADS
T (ng/ml)
200
150
100
50
0
0
50
100
150
200
250
Total DDT (ppm)
0
1
2
3
4
5
Pesticides (ppm)
10
20
30
40
PCBs (ppm)

Figure 7.

DDE (ppm)	5.3	5.3		0.9	0.8	0.7		0.3	0.2	0.1

Figure 8.